

WO02080951

Publication Title:

HERBAL EXTRACTS FOR THE TREATMENT OF CANCER

Abstract:

Abstract of WO02080951

The present invention is related to herbal extracts and their use as inhibitors of cancer cells lines in vitro and for the treatment of solid tumor cancer in humans. Data supplied from the esp@cenet database - Worldwide

Courtesy of <http://v3.espacenet.com>

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

**(19) World Intellectual Property Organization
International Bureau**



**(43) International Publication Date
17 October 2002 (17.10.2002)**

PCT

**(10) International Publication Number
WO 02/080951 A1**

(51) International Patent Classification ⁷ :	A61K 35/78	(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
(21) International Application Number:	PCT/US02/09937	
(22) International Filing Date:	29 March 2002 (29.03.2002)	
(25) Filing Language:	English	
(26) Publication Language:	English	
(30) Priority Data: 60/281,958	6 April 2001 (06.04.2001)	US
(71) Applicant (for all designated States except US): SYNER-GISTIX BIOTECH, INC. [US/US]; 2320 Woolsey Street, suite 100, Berkley, CA 94705 (US).		(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
(72) Inventor; and		
(75) Inventor/Applicant (for US only): COHEN, Isaac [US/US]; 2320 Woolsey Street, suite 100, Berkley, CA 94705 (US).		Published: — with international search report
(74) Agent: ROSE, Bernard, E; Lyon & Lyon LLP, 333 West San Carlos Street, suite 800, San Jose, CA 95110 (US).		For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published: — *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/080951 A1

(54) Title: HERBAL EXTRACTS FOR THE TREATMENT OF CANCER

(57) Abstract: The present invention is related to herbal extracts and their use as inhibitors of cancer cells lines in vitro and for the treatment of solid tumor cancer in humans.

HERBAL EXTRACTS FOR THE TREATMENT OF CANCER**SUMMARY OF THE INVENTION**

5 This invention relates to extracts of herbs where the extracts, when placed in contact with solid tumor cancer cells, inhibits the activity, that is, the growth and/or proliferation, of the cells. The extract may be an extract of one herb alone or it may be a combination of extracts of two or more herbs. The herbs are selected from among the species *Rhus chinensis*, *Acanthopanax gracilistylus*,

10 *Panax ginseng*, *Epimedium grandiflorum*, *Lithospermum erythrorhizon*, *Boswellia carterii*, *Commiphora myrrha*, *Lobelia chinensis*, *Lonicer iaponica*, *Stellaria dichotoma*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyi*, *Carthamus tinctoris*, *Rhaponticum uniflorum*, *Taraxacum mongolica*, *Eupolyphaga sinensis* (zoo.), *Luffa cylindrica*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Imperata cylindrica*, *Prunella vulgaris*, *Salvia chinensis*, *Scuttellaria barbata*, *Laminaria japonica*, *Caulis mutong*, *Gleditsia sinensis*, *Glycyrrhiza uralensis*, *Sophora japonica*, *Sophora flavescens*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Hibiscus mutabilis*, *Ligustrum lucidum*, *Daemonorops draco*, *Rheum palmatum*, *Radix clematidis*, *Ranunculus ternatus*, *Agrimonia pilosa*, *Duchesnea indica*,

15 *Eriobotrya iaponica*, *Heydyotis diffusa*, *Rubia cordifolia*, *Uncaria rhynchophylla*, *Salix babylonica*, *Sargassum pallidum*, *Solanum lyratum*, *Solanum melongena*, *Solanum nigrum* and *Sparganium stoloniferum*.

As is described in the Detailed Description section, below, some herbs are substantially more active than others in inhibiting the activity of cancer cells. It is therefore a presently preferred aspect of this invention that the herbal extract or extracts are obtained from the species *Panax ginseng*, *Lithospermum erythrorhizon*, *Boswellia carterii*, *Commiphora myrrha*, *Lonicer iaponica*, *Stellaria dichotoma*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyi*, *Carthamus tinctoris*, *Rhaponticum uniflorum*, *Taraxacum mongolica*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Salvia chinensis*, *Scuttellaria barbata*, *Caulis mutong*, *Gleditsia sinensis*, *Glycyrrhiza uralensis*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Ligustrum lucidum*, *Rheum palmatum*, *Agrimonia pilosa*, *Duchesnea indica*, *Eriobotrya iaponica*, *Rubia cordifolia* and *Uncaria rhynchophylla*.

It is a particularly presently preferred aspect of this invention that the herbal extract or extracts are obtained are of the species *Commiphora myrrha*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyl*, *Salvia chinensis*, *Scuttellaria barbata*, *Gleditsia sinensis*, *Anemarrhena*, *Viscum coloratum*, *Ligustrum lucidum*, *Rheum palmatum*, *Duchesnea indica*, *Eriobotra iaponica*, *Rubia cordifolia* and *Uncaria rhynchophylla*.

It is an aspect of this invention that the solid tumor cancer cell, the activity of which is inhibited by the herbal extract or extracts of this invention is an SKBR3 cell, an MCF7 cell, an MDA-MB231 cell, a BT474 cell or an MCNeuA cell.

A further aspect of this invention is a method for treating a solid tumor cancer, comprising administering to a patient a therapeutically effective amount of a composition comprising an extract of one or more of *Rhus chinensis*, *Acanthopanax gracilistylus*, *Panax ginseng*, *Epimedium grandiflorum*, *Lithospermum erythrorhizon*, *Boswellia carterii*, *Commiphora myrrha*, *Lobelia chinensis*, *Lonicer iaponica*, *Stellaria dichotoma*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyl*, *Carthamus tinctoris*, *Rhaponticum uniflorum*, *Taraxacum mongolica*, *Eupolyphaga sinensis* (zoo.), *Luffa cylindrica*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Imperata cylindrica*, *Prunella vulgaris*, *Salvia chinensis*, *Scuttellaria barbata*, *Laminaria japonica*, *Caulis mutong*, *Gleditsia sinensis*, *Glycyrrhiza uralensis*, *Sophora japonica*, *Sophora flavescens*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Hibiscus mutabilis*, *Ligustrum lucidum*, *Daemonorops draco*, *Rheum palmatum*, *Radix clematidis*, *Ranunculus ternatus*, *Agrimonia pilosa*, *Duchesnea indica*, *Eriobotra iaponica*, *Heydyotis diffusa*, *Rubia cordifolia*, *Uncaria rhynchophylla*, *Salix babylonica*, *Sargassum pallidum*, *Solanum lyratum*, *Solanum melongena*, *Solanum nigrum* and *Sparganium stoloniferum*.

It is a presently preferred aspect of this invention that the composition used to treat a patient comprises an extract or extracts from the herb species *Panax ginseng*, *Lithospermum erythrorhizon*, *Boswellia carterii*, *Commiphora myrrha*, *Lonicer iaponica*, *Stellaria dichotoma*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyl*, *Carthamus tinctoris*, *Rhaponticum uniflorum*, *Taraxacum mongolica*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Salvia chinensis*, *Scuttellaria barbata*, *Caulis mutong*, *Gleditsia sinensis*, *Glycyrrhiza uralensis*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Ligustrum lucidum*, *Rheum*

palmatum, Agrimonia pilosa, Duchesnea indica, Eriobotra iaponica, Rubia cordifolia and Uncaria rhynchophylla.

It is further a particularly presently preferred aspect of this invention that the extract or extracts used to treat a patient are obtained from the herb species

5 *Commiphora myrrha, Vaccaria segetalis, Sarcandra glabra, Artemisia argyl, Salvia chinensis, Scutellaria barbata, Gleditsia sinensis, Anemarrhena, Viscum coloratum, Ligustrum lucidum, Rheum palmatum, Duchesnea indica, Eriobotra iaponica, Rubia cordifolia and Uncaria rhynchophylla.*

The solid tumor cancer being treated is an epithelial cell cancer in another
10 aspect of this invention.

The epithelial cell cancer is breast or ovarian cancer in a still further aspect of this invention.

The patient being treated is a human being in yet another aspect of this invention.

15 An aspect of this invention is a composition comprising a pharmaceutically acceptable carrier or excipient and an extract or extracts of one or more of *Rhus chinensis, Acanthopanax gracilistylus, Panax ginseng, Epimedium grandiflorum, Lithospermum erythrorhizon, Boswellia carterii, Commiphora myrrha, Lobelia chinensis, Lonicera iaponica, Stellaria dichotoma, Vaccaria segetalis, Sarcandra glabra, Artemisia argyl, Carthamus tinctoris, Rhaponticum uniflorum, Taraxacum mongolica, Eupolyphaga sinensis (zoo.), Luffa cylindrica, Trichosanthes kirilowii, Dioscorea bulbifera, Imperata cylindrica, Prunella vulgaris, Salvia chinensis, Scutellaria barbata, Laminaria japonica, Caulis mutong, Gleditsia sinensis, Glycyrrhiza uralensis, Sophora japonica, Sophora flavescens, Anemarrhena asphodeloides, Viscum coloratum, Hibiscus mutabilis, Ligustrum lucidum, Daemonorops draco, Rheum palmatum, Radix clematidis, Ranunculus ternatus, Agrimonia pilosa, Duchesnea indica, Eriobotra iaponica, Heydyotis diffusa, Rubia cordifolia, Uncaria rhynchophylla, Salix babylonica, Sargassum pallidum, Solanum lyratum, Solanum melongena, Solanum nigrum and Sparganium stoloniferum.*

30 The composition comprises aqueous extracts of the above herb species in an aspect of this invention.

The composition comprises alcohol extracts of the above species in a further aspect of this invention. In a presently preferred embodiment of this invention, the alcohol used to extract the herbs is ethyl alcohol.

5 The composition comprises a combination of aqueous and alcohol extracts of the above species of herbs in still another aspect of this invention.

DETAILED DESCRIPTION OF THE INVENTION

Brief description of the Tables

Table 1 depicts herbs, from which extracts of this invention are obtained, listed by family, genus, species and traditional Chinese name, of this invention. In 10 addition Table 1 shows the dry weight amount of material obtained from each herb by aqueous extraction.

Table 2 shows the degree of inhibition of the activity of several solid cancer tumor cell lines by the extracts of this invention.

Brief description of the Figures

15 Figures 1A – 1F are dose-response curves showing the response of several solid cancer tumor cells to aqueous extracts of herbs of this invention.

Figure 2 depicts a gel electrophoresis plate which demonstrates that nuclear DNA disintegration occurs during apoptosis of solid tumor cancer cells in contact with aqueous extracts of herbs of this invention.

20 Definitions

As used herein, the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by, 25 practitioners of the chemical, pharmacological, biological, biochemical, medical and homeopathic arts.

As used herein, "inhibiting the activity" refers to slowing, preferably stopping, the growth and/or proliferation of cancerous cells, both in-place, i.e., growth and proliferation at the initial site of tumor formation, and proliferation by 30 metastasis. Inhibiting the activity also encompasses, in fact it is the most preferred embodiment of this invention, killing cancerous cells.

As used herein, the term "cancer" refers to various types of malignant neoplasms, most of which can invade surrounding tissues, and may metastasize

to different sites, as defined by Stedman's Medical Dictionary 25th edition (Hensyl ed. 1990). Examples of cancers which may be treated by the present invention include, but are not limited to, brain, ovarian, colon, prostate, kidney, bladder, breast, lung, oral and skin cancers. In a presently preferred embodiment of this
5 invention the cancer being treated is breast or ovarian cancer.

As used herein, the term "contacting" in the context of contacting a solid tumor cancer cell with an extract of this invention bringing an extract of this invention and a target cancer cell together in such a manner that the extract can affect the activity of the cell either directly or indirectly. As used herein, contacting
10 refers to procedures conducted in vitro, i.e. cancerous cells which are the object of this invention are studied, outside a patient. Cells existing outside the patient can be maintained or grown in cell culture dishes. For cells outside the organism, multiple methods exist, and are well-known to those skilled in the art, to contact extracts with cells including, but not limited to, simply placing cells in a broth
15 containing an extract of this invention, with or without employment of various well-known transmembrane carrier techniques and direct cell microinjection.

As used herein, an "extract" refers to the residue obtained after an herb, or selected part thereof is (1) for example, without limitation, chopped, crushed, pulverized, minced or otherwise treated to expose maximum surface area and (2)
20 is placed in intimate contact with a liquid, usually, but not necessarily, under conditions of agitation and elevated temperature. Then, after a period of time under the foregoing conditions, the mixture is filtered to remove solids and the liquid removed by, for example but not limitation, evaporation or freeze drying. The liquid used to obtain an extract may be water or an organic solvent, for
25 example, without limitation, an alcohol such as methyl, ethyl or isopropyl alcohol, a ketone such as acetone or methyl ethyl ketone (MEK), an ester such as ethyl acetate, an organochlorine compound such as methylene chloride, chloroform or carbon tetrachloride, a hydrocarbon such as pentane, hexane or benzene and the like. An extract may also be obtained by using a combination of these solvents
30 with or without water.

As used herein, an "herb" refers to any plant that is reputed to have medicinal value in Traditional Chinese Medicine (TCM). That is, the use of extracts of various parts of these plants have been passed down from ancient to

modern Chinese practitioners of homeopathy as a means for treating various ailments. In some instances, clinical evidence using standard Western medical research protocols have verified the utility of some of the extracts. While each of the herbs, and parts thereof, that make up the compositions of this invention have long been known in TCM, use of an extract or combination of extracts in a composition as disclosed herein for the treatment of solid tumor cancers, in particular breast and uterine cancer, has not been previously disclosed.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a solid tumor cancer and/or its attendant symptoms. In particular, the terms simply mean that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

As used herein, "administer," "administering" or "administration" refers to the delivery of an extract or extracts of this invention or of a pharmaceutical composition containing an extract or extracts of this invention to a patient in a manner suitable for the treatment of particular cancer being addressed.

A "patient" refers to any higher organism that is susceptible to solid tumor cancers. Examples of such higher organisms include, without limitation, mice, rats, rabbits, dogs, cats, horses, cows, pigs, sheep, fish and reptiles. Preferably, "patient" refers to a human being.

As used herein, the term "therapeutically effective amount" refers to that amount of an extract or combination of extracts of this invention which has the effect of (1) reducing the size of the tumor; (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis; (3) inhibiting to some extent (that is slowing to some extent, preferably stopping) tumor growth; and/or, (4) relieving to some extent (or preferably eliminating) one or more symptoms associated with the cancer.

As used herein, a "pharmaceutical composition" refers to a mixture of one or more of the extracts described herein with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmacological composition is to facilitate administration of an extract or extracts of this invention to a patient.

As used herein, a "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered composition.

As used herein, an "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an extract or extracts of this invention. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Discussion

At one time, botanical agents were the most significant group of substances used by healers to treat patients. According to a WHO survey, 80% of the world's population still relies heavily on herbal medicine as their primary source of therapy. In Western culture one-quarter of the active components of currently prescribed drugs were first identified in plants and over half of the 50 most popular drugs today are derived from plant materials. In addition, over 60% of chemotherapeutic agents used in the treatment of cancer are derived from natural substances.

A useful strategy for the discovery of biologically active compounds from plants is the ethno-pharmacological approach which uses information about traditional medicinal uses of plants. The long history of a plant's use in treating a disorder, regardless of whether the disorder is well-characterized, e.g., skin rash, or is rather more nebulous, e.g., hot blood, is a clear indicator that something in the plant has some manner of beneficial effect on a disorder, otherwise the use of the plant would have faded in time. Furthermore, the fact that homeopathic practitioners have been administering the plant or an extract thereof to human patients for, often, centuries provides a compelling argument for the safety of the plant or its extract in human beings.

Such alternative approaches to medicine are becoming more and more widely accepted and used in the United States as well to treat a broad spectrum of conditions as well as to maintain wellness. It is estimated that one in two Americans currently uses alternative therapies at one time or another. In particular, the most popular complementary or fully alternative approach to the treatment of their cancers by patients is botanical agents/herbal medicines.

Traditional Chinese medicine (TCM) is often the treatment modality of choice by cancer patients opting for an alternative approach to dealing with their ailment. Patients use TCM both as anti-cancer agents and to alleviate the side effects of standard chemotherapy. However, TCM lacks the scientifically sound methodology required of Western pharmacology and the use of TCM is often hit or miss in its effectiveness. There remains a need for the discovery of specific herbal extracts and combinations thereof that have a specific utility and for which there is scientific evidence as to why they work in that use. This invention provides such extracts and compositions thereof.

The herbal extracts of this invention were prepared standard decoction procedures well-known in the art. For example, without limitation, a chopped, ground, minced or pulverized portion, e.g., likewise without limitation, root, fruit, stem, sclerotium, rhizome or leaves, of a herb plant is simmered in water for a period of time. At the end of that period, the liquid is decanted off, giving a "tea" containing substances that are soluble in hot water. Table 1 shows the genus and species, as well as the Chinese name, of the herbs extracted and tested against a panel of solid tumor cancer cell lines. Table 1 also shows the dry weight of the water soluble component of the part of the herb extracted.

The effect of the above extracts on five cancer cell lines was evaluated.

The cell lines were four human breast cancer cell lines, SKBR3, MCF-7, MDA-MB231 and BT474, and one murine breast cancer cell line, MCNeuA.

Table 2 shows the inhibitory effect of the extracts. A (-) indicates that the extract inhibited the activity of that cell line from 0 to 25%, a (+) indicates that the extract inhibited the cell line by 26 to 50%, a (++) indicates about 51 to 75% inhibition while a (+++) indicates 76 – 100% inhibition of the activity of that cell line. As can be seen from Table 2, 17 of the extracts; i.e., those from *Epimedium grandiflorum*, *Commiphora myrrha*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyi*, *Taraxicum mongolici*, *Salvia chinensis*, *Scutellaria barbata*, *Gleditsia sinensis*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Ligustrum lucidum*, *Rheum palmatum*, *Duchesnea indica*, *Eriobotrya japonica*, *Rubia cordifolia* and *Uncaria rhynchophylla*, inhibited at least one cell line by from 76 to 100%.

While not being bound to any particular theory, an assay for DNA fragmentation when the cell lines were subjected to the extracts suggest that

inducement of apoptosis may constitute one mechanism by which the components of the extracts inhibit the activity of the cancerous cells. Using a procedure for the isolation of genomic DNA that allows for the analysis of both high and low molecular weight (HMW and LMW) DNA fragmentation during 5 apoptosis (Solovan, V. and Salminen, *A Rapid and Efficient Method for the Preparation of Genomic DNA Suitable for Analysis of Both High and Low Molecular Weight DNA Fragmentation during Apoptosis*, 1999, Brain Res. Protocols, 4:335-340), it was observed that all the inhibited cells underwent HMW DNA fragmentation. While low molecular weight ladders are produced only in 10 some apoptotic cells and not others, nearly all apoptotic cells display HMW DNA fragmentation (Walker, P. R. and Sikorska, M., *New Aspects of the Mechanism of DNA Fragmentation in Apoptosis*, 1997, Biochem. Cell Biol., 75:287-299). HMW DNA fragments are best resolved and analyzed using pulsed field gel electrophoresis. Although HMW DNA fragments are too large to be resolved by 15 conventional gel electrophoresis, they are able to leave the wells and enter conventional agarose gels, forming a compression band of about 20 to 50 kb, the upper limit of fractionation ability of these gels. (Solovan and Salminen, 1999; Walker and Sikorska, 1997). The results of this experiment for several of the most active extracts is shown in Figure 2. The control cells demonstrated little if any 20 fragmentation since the majority of the intact genomic DNA remained in the well (lane 10 in the upper panel and lanes 8 and 9 in the lower panel). The faint bands of DNA that did enter the gels in the control wells probably reflects a small percentage of naturally dying cells in those wells. The wells containing extracts, on the other hand, show substantial HMW DNA fragmentation, predominantly at 25 the 1:10 dilution, as evidenced by the significant fraction of DNA entering the gel but not being resolved and forming a compression band near the top of the gel.

Pharmaceutical Compositions and Modes of Administration

An extract of this invention can be administered to a patient either as such, that is, as a "tea" without combination with any other substances or further 30 manipulation or it can be administered as a pharmaceutical composition where the extract is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of the extract is administered. A therapeutically effective amount refers to that amount of the

extract that results in amelioration of symptoms or a prolongation of survival in a patient, and may include destruction of a malignant tumor or a microbial infection.

Toxicity and therapeutic efficacy of the extracts, i.e., determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population) can be determined by standard pharmaceutical procedures in cell cultures or experimental animals,. The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Extracts that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. In particular for internal use, the dosage of such extracts lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. On general, since all the extracts of this invention have been used in TCM, they are known, at least singly, and many times in combination, to be relatively non-toxic to humans and therefore it is expected that they will exhibit large therapeutic indices.

For any extract used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition and based on knowledge of TCM. (See e.g. Fingl *et al.*, in THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or organ dysfunction. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response is not adequate. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and

response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

If desired, standard western medicine techniques for formulation and administration may be used, such as those found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, to name just a few. Oral administration is the procedure most often used in TCM and is the presently preferred method of this invention.

For injection, an extract of this invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate an extract herein for use in the methods disclosed for the practice of the invention in dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, an extract of the present invention, in particular those formulated as solutions, may be administered parenterally, such as by intravenous injection. Likewise, an extract can be formulated, using pharmaceutically acceptable carriers well known in the art, into dosages suitable for oral administration. Such carriers enable extracts to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Pharmaceutical compositions suitable for use in the present invention are compositions wherein an extract is contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. A pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers including excipients and auxiliaries that facilitate processing of the extracts into preparations that can be used

- pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating,
- 5 dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of an extract in water-soluble form. Additionally, suspensions of an extract may be prepared as appropriate oily injection suspensions. Suitable

10 lipophilic vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that

15 increase the solubility of an extract to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining an extract with solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to

20 obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired,

25 disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or

30 titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of extracts and/or doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the extract in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the extract may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols.

5 Examples

The herbs from which the extracts of this invention were obtained were
10 purchased from Shen Nong Herbs, Berkeley, California. Their identity was confirmed by reference to traditional pharmacopoeia literature.

Preparation of extracts

Herbal extracts were prepared as "boiled teas," which is how most are prepared for use in traditional treatment regimes. Aqueous extracts were
15 prepared by adding 7.5 g of dry ground herb to 125 ml distilled water, bringing the mixture to a boil and then simmering for 45 minutes. The mixture was cooled, during which period most of the solids sank to the bottom of the vessel. The aqueous layer was carefully decanted off of the residual solids, centrifuged for 5 minutes at 1500 rpm, sterile filtered through a 0.45 µm filter and stored at 4° C
20 until used. Generally, the extracts were tested within 1 – 2 weeks of preparation although most of the active extracts were found to retain activity after storage at 4° C for several additional weeks. An aliquot of each extract was dried under vacuum and the dry weight of the water soluble substances extracted from each herb determined.

25 Cell lines and culture

The extracts were tested against four human breast cancer cell lines, SKBR3, MFC-7, MDA-MB231 and BT474, and one murine breast cancer cell line, MCNeuA. All lines were maintained in 90% DME supplemented with 2.0 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated
30 fetal bovine serum. Cells at 70 – 80% confluence were used for plating for growth inhibition assays.

In vitro inhibition of activity

Cells were plated in 96-well flat bottom plates at 5,000 to 10,000 cells/well. The difference in number of cells plated adjusts for differences in the growth rates of these cell lines. Cells were allowed to adhere to the well walls overnight then
5 the extracts were added to triplicate wells at a 1:10 final dilution in culture medium for initial screening. For generating dose-response curves, serial 3-fold dilutions, starting at 1:10 dilution over 6 rows of wells were used. Water was added to the control wells at 1:10 dilution in culture medium. The plates were incubated at 37° C, 5% CO₂, for 3 days and then assayed for growth inhibition using a crystal violet
10 assay (Bernhardt, G., et al., *Standardized Kinetic Microassay to Quantify Differential Chemosensitivity on the Basis of Proliferative Activity*, 1992, J. Cancer Res. Clin. Oncol., 118:35-43). Cells remaining adherent to the well walls were rinsed with PBS and with 1% glutaraldehyde in PBS for 15 minutes. After rinsing the wells one more time with PBS, the fixed cells were stained with 0.02%
15 aqueous crystal violet (50 µl/well) for 30 minutes after which the wells were washed thoroughly with distilled water. The crystal violet stain bound by the cells was solubilized in 79% ethanol (100 µl/well) and the plates analyzed on a microplate reader (Molecular Devices) at 595 nm. The percent inhibition was calculated as the average optical density of the control wells minus average
20 optical density extract well divided by the average optical density of the control wells. Dose-response curves on SKBR3, MCF7 and MCNeuA cells for several of the extracts are shown in Figure 1. As can be seen, the concentration at which the extracts inhibited the activity of the cells by 50% (the IC₅₀) ranged from over 1 mg/ml down to about 10 µg/ml.

25 Induction of apoptosis

To assay for DNA fragmentation as a marker of apoptosis, a procedure for the isolation of genomic DNA that allows for the analysis of both high and low molecular weight DNA fragmentation during apoptosis was used. MCNeuA cells were plated at 5X10⁵ cells/well in 6-well plates and allowed to adhere overnight.
30 Aqueous herbal extracts were added to each well at a 1:10 and a 1:50 dilution. Sterile water, diluted 1:10 in culture medium, was added to the control wells. After 24 hours, the cells were visually examined under a microscope and morphological

changes noted. Attached and floating cells were harvested, washed with cold PBS and embedded in agarose droplets. The agarose droplets containing the cells were incubated in lysis buffer (50 mM NaCl, 20 mM TrisHCl, pH 8.0, 20 mM EDTA, 0.5% sodium sarkosyl, 50 µg/ml RnaseA and 100 µg/ml proteinase K) for

- 5 1 hour at 37° C. The cells were then washed with PBS and distilled water and placed in the wells of a conventional 1% agarose gel and electrophoresed overnight at approximately 1 V/cm. The gels were then stained with ethidium bromide and photographed under UV transillumination to give inverse images.

The images obtained are shown in Figure 2.

10

CONCLUSION

Herbal extracts, their uses for the inhibition of solid tumor cancer cells and the treatment of such cancers in patients are described herein. Although certain embodiments and examples have been used to describe the present invention, it will be apparent to those skilled in the art that changes to the embodiments and 15 examples may be made without departing from the scope and spirit of this invention.

Other embodiments of this invention are contained within the following claims.

20

WHAT IS CLAIMED IS:

1. A method for inhibiting the activity of a solid tumor cell comprising contacting the cell with an extract of one or more herbs selected from the group consisting of *Rhus chinensis*, *Acanthopanax gracilistylus*, *Panax ginseng*,
5 *Epimedium grandiflorum*, *Lithospermum erythrorhizon*, *Boswellia carterii*,
Commiphora myrrha, *Lobelia chinensis*, *Lonicer iaponica*, *Stellaria dichotoma*,
Vaccaria segetalis, *Sarcandra glabra*, *Artemisia argyl*, *Carthamus tinctoris*,
Rhaponticum uniflorum, *Taraxacum mongolica*, *Eupolyphaga sinensis* (zoo.),
10 *Luffa cylindrica*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Imperata cylindrica*,
Prunella vulgaris, *Salvia chinensis*, *Scuttellaria barbata*, *Laminaria japonica*, *Caulis mutong*, *Gleditsia sinensis*, *Glycyrrhiza uralensis*, *Sophora japonica*, *Sophora flavesrens*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Hibiscus mutabilis*,
Ligustrum lucidum, *Daemonorops draco*, *Rheum palmatum*, *Radix clematidis*,
15 *Ranunculus ternatus*, *Agrimonia pilosa*, *Duchesnea indica*, *Eriobotra iaponica*,
Heydyotis diffusa, *Rubia cordifolia*, *Uncaria rhychophylla*, *Salix babylonica*,
Sargassum pallidum, *Solanum lyratum*, *Solanum melongena*, *Solanum nigrum*
and *Sparganium stoloniferum*.
- 20 2. The method of claim 1, wherein the one or more herbs are selected from the group consisting of *Panax ginseng*, *Lithospermum erythrorhizon*,
Boswellia carterii, *Commiphora myrrha*, *Lonicer iaponica*, *Stellaria dichotoma*,
Vaccaria segetalis, *Sarcandra glabra*, *Artemisia argyl*, *Carthamus tinctoris*,
Rhaponticum uniflorum, *Taraxacum mongolica*, *Trichosanthes kirilowii*, *Dioscorea*
25 *bulbifera*, *Salvia chinensis*, *Scuttellaria barbata*, *Caulis mutong*, *Gleditsia sinensis*,
Glycyrrhiza uralensis, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Ligustrum lucidum*, *Rheum palmatum*, *Agrimonia pilosa*, *Duchesnea indica*, *Eriobotra iaponica*, *Rubia cordifolia* and *Uncaria rhychophylla*.
- 30 3. The method of claim 1, wherein the one or more herbs are selected from the group consisting of *Epimedium grandiflorum*, *Commiphora myrrha*,
Vaccaria segetalis, *Sarcandra glabra*, *Artemisia argyl*, *Taraxicum mongolici*,
Salvia chinensis, *Scuttellaria barbata*, *Gleditsia sinensis*, *Anemarrhena*

asphodeloides, Viscum coloratum, Ligustrum lucidum, Rheum palmatum, Duchesnea indica, Eriobotra iaponica, Rubia cordifolia and Uncaria rhynchophylla.

4. The method of any of claims 1, 2 or 3, wherein the solid tumor cell is an SKBR3 cell, an MCF7 cell, an MDA-MB231 cell, a BT474 cell or an MCNeuA
5 cell.

5. A method for treating a solid tumor cancer, comprising administering to a patient in need thereof a therapeutically effective amount of a composition comprising an extract of one or more of *Rhus chinensis, Acanthopanax gracilistylus, Panax ginseng, Epimedium grandiflorum, Lithospermum erythrorhizon, Boswellia carterii, Commiphora myrrha, Lobelia chinensis, Lonicera iaponica, Stellaria dichotoma, Vaccaria segetalis, Sarcandra glabra, Artemisia argyi, Carthamus tinctoris, Rhaponticum uniflorum, Taraxacum mongolica, Eupolyphaga sinensis (zoo.), Luffa cylindrica, Trichosanthes kirilowii, Dioscorea bulbifera, Imperata cylindrica, Prunella vulgaris, Salvia chinensis, Scutellaria barbata, Laminaria japonica, Caulis mutong, Gleditsia sinensis, Glycyrrhiza uralensis, Sophora japonica, Sophora flavescens, Anemarrhena asphodeloides, Viscum coloratum, Hibiscus mutabilis, Ligustrum lucidum, Daemonorops draco, Rheum palmatum, Radix clematidis, Ranunculus ternatus, Agrimonia pilosa, Duchesnea indica, Eriobotra iaponica, Heydyotis diffusa, Rubia cordifolia, Uncaria rhynchophylla, Salix babylonica, Sargassum pallidum, Solanum lyratum, Solanum melongena, Solanum nigrum and Sparganium stoloniferum.*

6. The method of claim 5, wherein the composition comprises an
25 extract of one or more of *Panax ginseng, Lithospermum erythrorhizon, Boswellia carterii, Commiphora myrrha, Lonicera iaponica, Stellaria dichotoma, Vaccaria segetalis, Sarcandra glabra, Artemisia argyi, Carthamus tinctoris, Rhaponticum uniflorum, Taraxacum mongolica, Trichosanthes kirilowii, Dioscorea bulbifera, Salvia chinensis, Scutellaria barbata, Caulis mutong, Gleditsia sinensis, Glycyrrhiza uralensis, Anemarrhena asphodeloides, Viscum coloratum, Ligustrum lucidum, Rheum palmatum, Agrimonia pilosa, Duchesnea indica, Eriobotra iaponica, Rubia cordifolia and Uncaria rhynchophylla.*

7. The method of claim 5, wherein the composition comprises an extract of one or more of *Commiphora myrrha*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyl*, *Salvia chinensis*, *Scuttellaria barbata*, *Gleditsia sinensis*, *Anemarrhena*, *Viscum coloratum*, *Ligustrum lucidum*, *Rheum palmatum*,

5 *Duchesnea indica*, *Eriobotra iaponica*, *Rubia cordifolia* and *Uncaria rhynchophylla*.

8. The method of any one of claims 5, 6 or 7, wherein the solid tumor cancer is an epithelial cell cancer.

10 9. The method of claim 8, wherein the epithelial cell cancer is breast or ovarian cancer.

10. The method of claim 5, 6 or 7, wherein the patient is a human being.

15 11. A composition comprising:
a pharmaceutically acceptable carrier or excipient; and,
an extract or extracts of one or more of *Rhus chinensis*, *Acanthopanax gracilistylus*, *Panax ginseng*, *Epimedium grandiflorum*, *Lithospermum erythrorhizon*, *Boswellia carterii*, *Commiphora myrrha*, *Lobelia chinensis*, *Lonicera iaponica*, *Stellaria dichotoma*, *Vaccaria segetalis*, *Sarcandra glabra*, *Artemisia argyl*, *Carthamus tinctoris*, *Rhaponticum uniflorum*, *Taraxacum mongolica*,

20 *Eupolyphaga sinensis* (zoo.), *Luffa cylindrica*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Imperata cylindrica*, *Prunella vulgaris*, *Salvia chinensis*, *Scuttellaria barbata*, *Laminaria japonica*, *Caulis mutong*, *Gleditsia sinensis*, *Glycyrrhiza*,

25 *uralensis*, *Sophora japonica*, *Sophora flavescens*, *Anemarrhena asphodeloides*, *Viscum coloratum*, *Hibiscus mutabilis*, *Ligustrum lucidum*, *Daemonorops draco*, *Rheum palmatum*, *Radix clematidis*, *Ranunculus ternatus*, *Agrimonia pilosa*, *Duchesnea indica*, *Eriobotra iaponica*, *Heydyotis diffusa*, *Rubia cordifolia*, *Uncaria rhynchophylla*, *Salix babylonica*, *Sargassum pallidum*, *Solanum lyratum*, *Solanum melongena*, *Solanum nigrum* and *Sparganium stoloniferum*.

30 12. The method of one of claims 1, 5 or 12, wherein the extract is an aqueous extract.

13. The method of any one of claims 1, 5 or 12, wherein the extract is an alcohol extract.

5

14. The method of claim 13, wherein the alcohol is ethyl alcohol.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/09857

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 13 and 14 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/09987

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 35/78
US CL :424/725, 728, 195.18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/725, 728, 195.18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----	Database JPAB on West, JP 63201130 A (USHIO), 19 August 1988, Abstract.	1, 5, 10-12 ----
Y		4, 8, 9
X ----	Database DWPI, Accession No. 1240147 (LI), 05 January 2000, Abstract.	1, 2, 5, 6, 8-12 ----
Y		4
X ----	US 5,919,821 A (SIMMET et al) 06 July 1999, see entire document, especially col. 2, line 66 - col. 3, line 21.	1, 2, 5, 6, 10-12 ----
Y		4, 8, 9
Y	US 6,039,949 A (PERO) 21 March 2000, see entire document.	1-12

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
"*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
21 MAY 2002	12 JUL 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20251 Facsimile No. (703) 305-9230	Authorized officer <i>Valerie Bell-Harris, Jr.</i> CHRISTOPHER TATE Telephone No. (703) 308-0196